Research Article

Statistical analysis of heavy metals in *Catlacatla*, *Wallago attu* and *Tilapia nilotica* fish at head Sulemanki in Satlujriver

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Abstract

The present study was aimed to evaluate the concentration of heavy metals in various organs of three edible fish species (*Catlacatla*, Wallagoattu and *Tilapia nilotica*) sampled from contaminated water of River Setluj at head Sulemanki (Okara), Pakistan. The concentration of selected heavy metals iron (Fe), nickel (Ni), Chromium (Cr), Cadmium (Cd) and lead (Pb) are compared in skin muscles, heart, liver, kidney and gills of same fish and also made comparison among three type of fishes. Overall absorption of heavy metals in three type of fishes is as follow Fe >Pb> Ni > Cr > Cd. The concentration of heavy metals in all three species of fishes is compared as *Tilapia nilotica > Catlacatla > Wallagoattu*, while the existence of heavy metals in the organs of fish is also compared, kidney > liver > gills > skin muscles > heart. In analysis of variance accumulation of heavy metals in three species is significantly present, as p-value is less than 0.05 for all heavy metals. Measured elevated levels of metal concentrations in fish organs is pointed out to be risks for potential health of fish, other aquatic organisms and human health.

Keywords: *Catlacatla*; Heavy metal; organs; *Tilapia nilotica* and Wallagoattu

Introduction

With the increase in human population the demand for the food supply has increased. The requirement of fish and shell fish has much increased because they are the excellent supply of protein. Globally men get 25% protein from fish and shell fish [1]. Fish also has a rich resource of vitamins and minerals which are good for human health [2]. In 2004 nearly 75% global fish was used for straight human utilization, however in 1997 the demand was 57% and it will exceed to 98.6% in 2020 [3]. Metal concentration has both valuable and damaging effects on living organisms. A few of these metals are very injurious in a very little quantity while some biologically important metals are harmful at high concentration. Intake of heavy metals in surplus, reacts with bio element (these elements are necessary for the functioning of life) present in body and destroy the structure of important molecules which take part in many metabolic reactions of body [4]. Heavy metals word is applied to group of metals belonging to metalloids that have atomic gradient greater than $4g/cm^3$ or five times greater than water. These are also

called trace elements as they are present in very little quantity in natural system [5]. Bioaccumulation of trace elements causes serious health problems and disturbs the environment through food web. Deposition of these elements in body organs particularly in the fish muscles, upset not only the growth and reproduction but also tissues level; caused changes in skin, gills and kidney. Metal deposition in humans causes serious effect on the functioning of liver, kidney, brain and muscle [6].

Aquatic flora and fauna has been polluted, trace element has become the world's major dilemma. These elements are entering the aquatic ecosystem by various sources like use of insecticides, pesticides, fertilizers and leaching [7]. Trace elements are degraded by human body [8]. They are deposited in different organs of the body and make threats to human health. So these trace elements are among the hazardous contaminant that's why they are included in unsafe class of pollutants in river and seas [9]. One of the incidents occurred in the surrounding area of Japanese fishing harbor the Minimata disease which was the result of eating fish and shrimps. They were polluted with methyl mercury and non-organic mercury, the effluents released by the factories [10].

From the last two decades estimation of heavy metals in fish has been increased in order to provide the safe food to humans and to reduce the injurious effects of these metals. Heavy metals have high molecular weight and poisonous in small amount. These metals may build up in living creature when they are taken in and then deposited quickly in body. They cannot metabolize easily. They enter into the water by industrial waste and user's material. Acid rain also plays a role in breaking down the soil and leach trace metals into the water (streams, rivers, lakes and ground water. Trace elements bioaccumulation may occur in the fishes and other animals. Bioaccumulation means deposition of poisonous elements in the body organs with passage of time. Generally heavy metals are divided into two classes vital and non-vital. The non-vital elements includes: Al, Hg, Cd, Sn and Pb and have no biochemical activity and their toxic effect increases with the increase in concentration. Vital elements includes: Cu, Zn, Cr, Ni, Co, Mo and Fe. Food is the main path for taking these heavy metal ions. Fish is also the element of this diet; it is not shocking that some of the heavy metals pass to human beings through contaminated fish [11]. The purpose of the present study is to illustrate the comparison of heavy metals, Fe (Iron), Ni (Nickel), Cr (Chromium), Cd (Cadmium) and Pb (Lead) concentration in the different organs of *Catlacatla*, *Wallagoattu* and *Tilapia nilotica* fish.

In recent years, there is an increasing tendency to check effects of dense metals on aquatic biota especially the fishes because it is a staple diet of humans in some countries, being a part of food chain, it should be free of pollutants. Marine water is often checked for heavy metal contamination in rivers and streams, and its ground surface and organisms. Investigation of heavy metals in body cells is the big source for establishing public health standard. The Arabian Gulf was the center of attraction for research in recent past years because of accidental oil spill in 1991. So the heavy metals storage was checked in marine fish Epinephelus micro don. They also act as pollution indicator for marine life and other organisms [12]. Examination of dense metals on River Ravi made by the researchers by observing the heavy metal contents in gills, liver, kidneys, fins, scale, skin and bones of fishes *Labeorohita*, *Catlacatla* and Cirrhiniamrigala. Quantity of heavy metals was varying because it was based on the season for collection of the samples. Deposition of heavy metals was high in vital organs and less in muscle and fat. River Ravi has lot of heavy metals as reported by the EPA. Through wet digestion process dense metals concentration was determined by absorption spectrometry. Heavy metal have

tenacity in surface water, found in colloidal and particulate form in rivers, and is considered as prominent way for the transportation of metals [13]. Anthropogenic activities have serious effect on aquatic life. Normally metals are required for healthy life activities in low quantity. But the heavy metals have lethal effects for aquatic biota especially for the fishes. In fishes the entrance of these metals is through body surface, gills and the digestive tract. Food may be the one factor by which metal deposition occurs in the body. One of the major threats to fish life is the industrial effluents and domestic sewage, released in to the freshwater bodies. Heavy metal deposition is checked on fishes of River Ravi and its related tributaries. Fishes were collected from Baloki head works and Sindhnai barrage. Heavy metal concentration was analyzed by SMEWW method, after doing wet digestion, sample analysis is done though Atomic absorption spectrophotometer. Standing water is quite different from the flowing water. Its depth, flowing rate, geology and turbidity are totally different; contaminants enter the river, flow downstream but their concentration cannot be changed. Huge amount of industrial waste and sewage water are released into the river through tributaries which changes quality of river bed sediments [14]. When stress condition (manganese) were applied to fishes, show great weight loss. Stress has a profound effect on metabolism of fishes and so reduction in growth was observed in freshwater fishes [15]. Heavy metals absorbed in the body of fish through three ways: gills, body surface and digestive track. Level of heavy metals was checked in different organs of freshwater fish that have direct contact with polluted water. It was found that absorption cadmium and lead is high in tissues than other heavy metals, this level is raised because fish is first consumed in aquatic biota. The chromium absorption in rivers and lakes is about one to ten micro gram per liter while the EPA has set the level up to 50 to 100

microgram per liter for human safety and aquatic life. Chromium has a lethal effect on kinds of fish's effect on blood result in anemia, eosiophilia, lymphocytosis and bronchial and renal wounds [16]. Different heavy metals concentration is determined in the organs of fish Tilapia (*Oreochromisniloticus*). Heavy metals are the major contaminants that enter our body through food. Some metals bound to body tissues such as cadmium bind to kidneys and lead to bones. The measurement is done through inductively-coupled plasma mass spectrometry. By this method zinc absorption was estimated. Study showed that concentration of heavy metal is high in liver, than gills and muscles. It was noted that metabolite region of body like liver, gills and kidney are suspected to have high metal absorption than muscles [17]. To estimate the outcome of heavy metals (Fe, Cu, Cr and Pb) on the tissue organs of fish species Cyprinuscarpioand, *Wallago attu* their samples were collected from Indus river their deposition in gills, kidney, liver and muscles is matched up to International standard of food fish. There is noteworthy dissimilarity in the deposition of heavy metals in the visceral organs of the two fishes. Each metal is with tolerable level as mentioned by International authorities like World health organization, FAO and FEPA [18].

Materials and methods

The study was carried out the fishing of Sutlej River at head Sulemaniki District Okara, which is located in the south-east of the Punjab, Pakistan. The residents of District Oakara and District Bahawalnagar are dependent on the Sutlej River for fishing and fish consumption. In this area, an important issue is to check the quality of fish meat with regards to heavy metals in organs of fish. Sutlej river inter in Pakistan at District Qasor from India with heavy metals discharge due to industrialization and urbanization greatly affect the aquatic life. Fish is an important member of aquatic food chain and is sensitive to heavy metal. Fish is not only the pollution indicator of

aquatic ecosystem but also the main sources of proteins in human nutrition. For the examination of heavy metals three species *Catlacatla*, Wallagoattu and *Tilapia nilotica* of adult fishes were taken at head sulemaniki.

After identification, fishes were washed with deionized water, sealed in polythene bags and kept in freezer at -20ºC until used for chemical analysis. Then three samples of each tissue (liver, gills, muscles, kidney and heart) from each species of fish were dissected. The representative samples were cautiously sealed in tagged sampled bottles containing aqueous Bouin's fixative for 24 hours, and sent to the laboratory at Mycology department of Botany, University of Punjab, Lahore for the examination of heavy metals for Fe, Ni, Cr, Cd, and Pb.

One gram of each wet fish tissues (liver, gills and kidney, heart and skin muscles) was digested with 2ml of HNO_3 and 1ml of H^2SO_4 in a volume metric flask, digestion was carried out on a hot plate (200^0C to 250^0C) until transparent or vine green color was obtained. The digested solution was diluted up to 100ml and from this 50ml of each sample is taken. Each sample was filtered through 0.42-micro meter filter paper; finally each sample was transferred to polythene bottle and sealed to avoid contamination and evaporation. All digested samples are stored at 4^0C before heavy metal analysis [19].

All reagents were of analytical grade. Double deionized water was used for all digestion. HNO_3 and H_2SO_4 were of supra-pure quality (Merck, Germany). All glassware were cleaned by soaking in dilute HNO_3 (10%) and rinsed with deionized water before to use. For each element, standard solution used for calibration (sigma) is prepared by diluting the stock solutions to the concentration of 1000 mg/L. Standard reference materials are used [19].

For the observed data mean values, standard error and analysis of variance were calculated for heavy metals (Fe, Ni, Cr, Cd and Pb) and tissues (kidney, liver, skin muscles, heart and gills) of each fish species. Data were analyzed by using Statistical package SPSS 21 at level of significance was P<0.05.

Results and discussion

In table 1 water sample at head sulemanki has 0.1410±0.01674 mg/l of fe, while the amount of iron in *Catlacatla*, *Wallago attu* and *Tilapia nilotica* fish is 1.8751±0.28851, 1.5285±0.06232 and 2.4517±0.53667 which shows *Tilapia nilotica* has more amount of fe than other two species of fishes. Amount of Ni in *Tilapia nilotica* fish is more than other two type of fishes and in water sample Ni is 0.0273±0.00203 mg/l. While amount of Cr, Cd and Pb in these types of fishes is compared as *Wallagoattu > Catlacatla > Tilapia nilotica* , *Wallago attu > Tilapia nilotica > Catlacatla* and *Tilapia nilotica> Catlacatla > Wallago attu*.

Critical analysis of Iron (Fe): in table 2, 3 and 4, concentration of iron in the organs of *Catlacatla* fish is ranges from 3.9090 ± 0.14271 to 0.7113 ± 0.00606, in *Wallago attu* ranges from 1.7557 ± 0.00644 to 1.1127 ± 0.00467 and *Tilapia nilotica*fishis from 6.1277 ± 0.00970 to 0.0320 ± 0.00351 mg/l. Comparing the amount of iron in different organs of these fish species we found thatin skin muscles*Catlacatla > Tilapia nilotica >Wallago attu*, in heart *Wallago attu > Catlacatla > Tilapia nilotica*, in liver *Catlacatla > Tilapia nilotica > Wallago attu*, in kidney*Tilapia nilotica > Wallago attu> Catlacatla* and in gills *Tilapia nilotica > Wallago attu > Catlacatla*. The value of F- statistics (967.804) in ANOVA table 5 for iron is significant as p-value is less than 0.05, which shows that a considerable amount of heavy metal iron is detected in all samples of each type of fish.

Comparing Nickel (Ni): in table 2, 3 and 4, existence of heavy metal nickel in the organs of *Catlacatla* fish is ranges from 0.4553±0.00384 to 0.0867±0.00882, in *Wallago attu* ranges from 0.3443 ± 0.01501 to 0.1440 ± 0.01179 and in *Tilapia nilotica*

fish is from 0.3550 ± 0.00379 to 0.1877 ± 0.00825 mg/l. Comparing the amount of nickel in different organs of three fish species we found that in skin muscles *Tilapia nilotica* > *Catlacatla* > *Wallago attu*, in heart *Wallago attu* > *Catlacatla* > *Tilapia nilotica*, in liver *Catlacatla* > *Wallago attu* > *Tilapia nilotica* , in kidney*Tilapianilotica* > *Catlacatla*> *Wallago attu* and in gills *Tilapia nilotica* > *Wallago attu* > *Catlacatla*. In ANOVA table 5 the value of F-test for nickel is 99.52 and its p-value is 0.0000, which shows that nickel occur significantly in all samples of each type of fish.

Comparing Chromium (Cr): in table 2, 3 and 4, presence of the amount of heavy metal Chromium in the organs of *Catlacatla* fish is ranges from 0.06075±0.00067 to 0.02003±0.00404, in *Wallago attu* ranges from 0.0803 ± 0.00067 to 0.0277 ± 0.00088 and in *Tilapia nilotica* fishis from 0.0413 ± 0.00067 to 0.0137 ± 0.00203 mg/l. While comparing the amount of Chromium in different organs of these fish species we explore that in skin muscles*Tilapia nilotica*> *Wallago attu* > *Catlacatla*, in heart *Wallago attu* > *Catlacatla* > *Tilapia nilotica*, in liver *Wallago attu* > *Tilapia nilotica* > *Catlacatla*, in kidney *Catlacatla* > *Wallago attu*> *Tilapia nilotica* and in gills *Tilapia nilotica* > *Wallago attu* > *Catlacatla*. In ANOVA table 5 the value of F-test for Chromium is 102.097 and its p-value is less than 0.05, which shows that a significant amount of Chromium is detected in all samples of each type of fish.

Discussing the metal Cadmium (Cd): from table 2, 3 and 4, the Heavy metal Cadmium in the organs of *Catlacatla* fish is ranges from 0.1020±0.00577 to 0.0000±0.0000, in *Wallago attu* ranges from 0.2517 ± 0.01785 to 0.0223 ± 0.00186 and in *Tilapia nilotica* fish is from 0.1360 ± 0.00971 to 0.0487 ± 0.00260 mg/l. Comparing the amount of Cadmium in different organs of these fish species we found that in skin muscles*Wallagoattu* > *Catlacatla* > *Tilapia nilotica*, in heart *Tilapia nilotica* > *Wallago attu* > *Catlacatla*, in liver *Wallago attu* > *Catlacatla* > *Tilapia nilotica*, in kidney*Catlacatla* > *Wallago attu* > *Tilapia nilotica* and in gills *Tilapia nilotica* > *Catlacatla* > *Wallago attu*. The value of F-test for Cadmium is 58.77 and its p-value is less than 0.05, which shows that a significant amount of Cadmium is found in all samples of each type of fish.

Comparing Lead (Pb): in table 2, 3 and 4, the presence of the amount of heavy metal Lead in the organs of *Catlacatla* fish is ranges from 1.0043±0.07738 to 0.1477±0.01333, in *Wallago attu* ranges from 0.6617 ± 0.7448 to 0.3750 ± 0.03743 and in *Tilapia nilotica* fish is from 1.0367 ± 0.06282 to 0.3297 ± 0.02973 mg/l. Comparing the amount of Lead in different organs of these fish species we found that in skin muscles *Catlacatla* > *Wallago attu* > *Tilapia nilotica*, in heart *Tilapia nilotica* > *Wallago attu* > *Catlacatla* in liver *Catlacatla* > *Wallago attu* > *Tilapia nilotica* , in kidney *Tilapianilotica* > *Catlacatla* > *Wallago attu* and in gills *Catlacatla*> *Wallago attu* > *Tilapia nilotica*. In ANOVA table 5 the value of F-test for Lead is 29.747 and its p-value is less than 0.05, which shows that a significant amount of Lead is present in all samples of fishes.

Table 1. Descriptive statistics of heavy metals in three fish species

Metals	N	*Catlacatla* ($\overline{X} \pm s_{\overline{x}}$)	*Wallago attu* ($\overline{X} \pm s_{\overline{x}}$)	*Tilapia nilotica* ($\overline{X} \pm s_{\overline{x}}$)	Water ($\overline{X} \pm s_{\overline{x}}$)
Fe	15	1.8751±0.28851	1.5285±0.06232	2.4517±0.53667	0.1410±0.01674
Ni	15	0.2531±0.3244	0.2235±0.02035	0.2663±0.01552	0.0273±0.00203
Cr	15	0.0351±0.00391	0.0441±0.00548	0.0273±0.00260	0.0113±0.00067
Cd	15	0.0600±0.00926	0.0947±0.02223	0.0778±0.00847	0.0617±0.00581
Pb	15	0.5435±0.07696	0.5070±0.03813	0.5663±0.07015	0.0983±0.00498

Table 2. Descriptive statistics of heavy metals in organs of *Catlacatla* fish

Organs	N	Fe ($\bar{X} \pm s_{\bar{x}}$)	Ni ($\bar{X} \pm s_{\bar{x}}$)	Cr ($\bar{X} \pm s_{\bar{x}}$)	Cd ($\bar{X} \pm s_{\bar{x}}$)	Pb($\bar{X} \pm s_{\bar{x}}$)
Skin Muscle	3	1.7307±.02074	.2107±.00593	.0310±.00153	.0520±.00764	.6033±.00333
Heart	3	.7113±.00606	.2260±.01950	.0390±.00153	.0000±.00000	.1477±.01333
Liver	3	3.9090±.14271	.4553±.00384	.0247±.00145	.0717±.00426	.5760±.02219
Kidney	3	1.5033±.01534	.2867±.01384	.0607±.00067	.1020±.00577	.3860±.03724
Gills	3	1.5210±.06655	.0867±.00882	.0200±.00404	.0743±.00285	1.0043±.07738

Table 3. Descriptive statistics of heavy metals in organs of *Wallago attu* fish

Organs	N	Fe ($\bar{X} \pm s_{\bar{x}}$)	Ni ($\bar{X} \pm s_{\bar{x}}$)	Cr ($\bar{X} \pm s_{\bar{x}}$)	Cd ($\bar{X} \pm s_{\bar{x}}$)	Pb($\bar{X} \pm s_{\bar{x}}$)
Skin	3	1.1127±.00467	.1477±.00825	.0317±.00120	.0940±.01137	.3667±.02801
Muscle	3	1.6520±.01185	.3443±.01501	.0527±.00318	.0423±.00186	.6617±.07448
Heart	3	1.4507±.04864	.2640±.00603	.0803±.00067	.2517±.01785	0.5073±.07389
Liver	3	1.7557±.00644	.2173±.00913	.0277±.00088	.0630±.00551	0.3750±.03743
Kidney	3	1.6713±.01543	.1440±.01179	.0280±.00173	.0223±.00186	.6243±.01697
Gills						

Table 4. Descriptive statistics of heavy metals in organs of *Tilapia nilotica* fish

Organs	N	Fe ($\bar{X} \pm s_{\bar{x}}$)	Ni ($\bar{X} \pm s_{\bar{x}}$)	Cr ($\bar{X} \pm s_{\bar{x}}$)	Cd ($\bar{X} \pm s_{\bar{x}}$)	Pb($\bar{X} \pm s_{\bar{x}}$)
Skin Muscle	3	1.6210±.04080	.2337±.01889	.0413±.00067	.0487±.00260	.3297±.02973
Heart	3	.0320±.00351	.1877±.00825	.0213±.00067	.0730±.00462	1.0367±.06282
Liver	3	2.2537±.06443	.2630±.00874	.0270±.00153	.0697±.00984	.3687±.01408
Kidney	3	6.1277±.00970	.2923±.00393	.0137±.00203	.0617±.00088	.5370±.06612
Gills	3	2.2243±.01202	.3550±.00379	.0330±.00173	.1360±.00971	.5593±.06502

Table 5. ANOVA Table for the heavy metals in the organs of fishes

		Sum of Squares	Df	Mean Square	F	Sig.
Fe	Between Groups	94.319	15	6.288	967.804	.000
	Within Groups	.208	32	.006		
	Total	94.526	47			
Ni	Between Groups	.500	15	.033	99.525	.000
	Within Groups	.011	32	.000		
	Total	.511	47			
Cr	Between Groups	.014	15	.001	102.097	.000
	Within Groups	.000	32	.000		
	Total	.015	47			
Cd	Between Groups	.142	15	.009	58.770	.000
	Within Groups	.005	32	.000		
	Total	.147	47			
Pb	Between Groups	2.944	15	.196	29.747	.000
	Within Groups	.211	32	.007		
	Total	3.155	47			

Conclusion

Absorption of heavy metals in three fish species are Fe > Pb > Ni > Cr > Cd. The concentration of heavy metals in all three species of fishes is compared as *Tilapia nilotica* > *Catlacatla* > Wallagoattu, while the existence of heavy metals in the organs of fishes is also compared, kidney > liver > gills > skin muscles > heart. Accumulation of all heavy metals is significantly present in the samples of the species of fish.

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